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THE EFFECTS OF GAS SWITCHING DURING DECOMPRESSION ON DECOMPRESSION SICKNESS IN A SWINE MODEL

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13. ABSTRACT *(Maximum 200 words)*

In a porcine model of neurological and cutaneous decompression sickness (DCS), the effect of switching the inert gas to nitrogen during decompression on a heliox dive was examined. Control pigs were dived in a dry chamber environment on a profile of 250 feet of seawater (fsw) for 30 min with 3 decompression stops, 10 min at 120 fsw, 20 min at 60 fsw, and 50 min at 20 fsw. The experimental group underwent the same dive except the breathing mixture was switched to air at 170 fsw. Each group comprised 32 pigs. Animals were observed postdecompression for the onset of neurological and cutaneous DCS. In the control group, 20 pigs developed neurological DCS and 12 manifested cutaneous DCS. In the experimental group, 14 pigs developed neurological DCS and 4 developed cutaneous DCS. The difference in incidence for neurological DCS (0.6 vs 0.4) was not significant using chi-square analysis with Yates' correction (χ^2=1.57; $p > 0.10$), but was smaller than the detection limit of the study design. The difference in incidence for cutaneous DCS (0.4 vs 0.1) was significant ($\chi^2 = 4.08$; $p < 0.05$). The results suggest a slight benefit to gas switching in pigs during decompression from a helium/oxygen (heliox) dive of this depth and duration.

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The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, National Research Council, National Academy Press, 1996.

INTRODUCTION

A possible benefit to switching gas during decompression was first suggested by Keller and Buhlmann in 1965 based on a small series of human dives (1). In that work, the authors discussed the theoretical benefits of switching to a heavier gas such as nitrogen (N_2) during decompression from a short, deep dive on a lighter gas, such as helium (He). They hypothesized that since lighter gases diffuse into and out of the body more quickly than heavier gases, replacing the breathing mixture with a heavier, slower gas during decompression allows a shortening of decompression time. The lighter gas is quickly eliminated from the tissues not only because of its more rapid diffusion but also because of the large gradient created between the blood and the alveolar gas tension when the breathing mixture is switched. This rapid off-gassing can occur without any significant uptake of N_2 into the tissues, because its diffusion is much slower and most of the decompression time is at shallower depths. These theoretical benefits were subsequently demonstrated in rats by Lillo and MacCallum in a controlled series of experiments demonstrating the effect of gas switching with gases of different potencies ($He<N_2<Ar$) for causing decompression sickness (DCS) and of different exchange rates ($He>Ar>N_2$) (2). In the rat study, animals were explosively decompressed at 5, 15, and 25 min after the gas switch. In each case the incidence of DCS for the group switched to air was less than that for the group that remained on $He\text{-}O_2$.

Despite these observations and the fact that gas switching is used routinely in commercial diving, the human data concerning gas switching remains scant. No human study to date has been controlled to exclude changes other than the shift to N_2. Thalmann produced the most complete examination of humans in a series of 0.7 ATA helium/oxygen (heliox) dives in which

air was substituted for the heliox mixture during decompression (3). He found no significant difference in DCS incidence. However, the diving apparatus used allowed the PO_2 to decrease at stops shallower than 80 feet of seawater (fsw) where the switch was made. The changing partial pressure of oxygen potentially confounds the results.

There exists, therefore, a discrepancy between the small animal data and the limited human data on the benefit of gas switching. The discrepancy may be an important one in military and commercial diving operations where deep heliox diving is performed, requiring very long decompression times. The following study was carried out to investigate this discrepancy using an established, porcine model of neurological DCS (4-6).

METHODS

Subjects and Animal Husbandry

Juvenile, male, neutered, pure-bred, Yorkshire swine from a closed breeding colony (weight range 16-20 kg on delivery) were received as numbered littermates. On receipt, pigs were examined by a veterinarian and each fitted with an adjustable canine chest harness (Coastal Pet Products, Alliance, OH) to facilitate handling. All pigs were housed in pairs until catheterized, at which time they were separated and placed in different run indoors. Water was freely available in each run and the minimum daily diet consisted of 2% by body weight of Purina Hog Finisher No. 50, which maintained a gradual weight increase with growth.

Pigs were in-house for about one week before diving. After an initial 24 h to adjust to their new surroundings, the animals were introduced into the laboratory environment. Each weekday they were transported from the animal care facility to the laboratory in plastic transport

kennels (Vari-Kennel, R.C. Steele, Brockport, NY). All pigs were then habituated to the general laboratory conditions and to the 4 ft x 4 ft enclosure where they would be observed for signs of DCS after diving. Each pig was also familiarized with the compression chamber and with the noise of flowing gas experienced during the dive.

All pigs were trained to run on a modified laboratory treadmill (Marquette Electronics, Milwaukee, WI). Training was made easier if a novice pig first observed an experienced pig running on the treadmill. The best time for training was before the morning feeding, as gastro-colic reflex effects were reduced and feeding immediately after the treadmill session induced a Pavlovian response to training. After 3 - 4 sessions, most pigs ran easily on a 5% incline at a speed of 4 mph for 5 min. More prolonged or strenuous training was avoided because physical conditioning had been found to reduce the risk of neurological DCS (6).

Predive Preparation

Procedures were carried out with pigs passively restrained in a Panepinto sling (Charles River; Wilmington, MA), which cradled the animals' body while the legs hung down through openings. The slings were mounted on wheeled carts, permitting easy movement around the laboratory.

On the afternoon before their dive, pigs were anesthetized (IM ketamine, 400 mg; IM xylazine, 20 mg) and venous catheterization of an ear vein was performed. This enabled both venous blood sampling predive and rapid venous access if a pig developed DCS after diving. We used customized, 18-inch long, polyurethane catheters (Braintree Scientific, Inc., Braintree, MA) with 0.040-inch external / 0.025-inch internal diameter and an integral luer hub. After a cut-

down onto the ear vein, the sterilized catheter was advanced 10 - 12 inches into the central thoracic veins, then lightly tied into the vessel.

The cut-down incision was sutured closed and an injection port (Interlink System, Baxter, Deerfield, IL) was fitted to the catheter. Pigs were then given IV chloramphenicol, 500 mg, to reduce the risk of infection, and then the injection port was heparinized to maintain catheter patency overnight. The catheter was then firmly secured to the dorsum of the pig's ear using 2-inch woven, surgical, adhesive tape (National Patent Partnership, Dayville, CT). This type of tape proved the most reliable in confounding the pigs' attempts to remove it.

Before diving, each pig fasted overnight but had access to water *ad libitum*. In the morning, the pig was weighed, then placed in a Panepinto sling. The IV catheter was untaped and predive blood samples were drawn. The catheter was then re-taped to the ear. Handled gently, pigs tolerated these predive procedures with no or minimal complaint, making sedation or anesthesia unnecessary.

Dive Procedures

All pigs were dived once only, as described below. The compression chamber was a 66 inch x 30 inch cylinder (Bethlehem Corporation, Baltimore, MD). Each pig was dived while unrestrained in a transport kennel. The compression profile was controlled automatically by a computerized unit (Digital Programmer, Honeywell Corp., Phoenix, AZ) that responded to a pressure transducer in the chamber (Smart Transmitter, 900 Series, Honeywell Corp.) with automated control compression and exhaust valves (SVF, Santa Anna, CA). The decompression profile was guided manually to follow a preprogrammed track displayed by a computerized unit.

This displayed a real-time, display calibrated as fsw off-track. An accuracy of within ± 2 fsw off-track was consistently achieved during the decompression.

Control Dives

A total of 32 pigs performed a dive on 80%/20 % heliox (see Figure 1). The dive was to 250 fsw (765.8 kPa) for 30 min. Compression took 5 min 50 s: 2 min at 20 fsw/min (61 kPa/min) to 40 fsw; 1 min at 40 fsw/min (122 kPa/min) to 80 fsw; then 2 min 50 s at 60 fsw/min (183 kPa/min) to 250 fsw. Time at chamber bottom was 24 min 10 s, followed by decompression at 60 fsw/min with decompression stops of 10 min at 120 fsw, 20 min at 60 fsw, and 50 min at 20 fsw. Total decompression time (TDT) was 1 h, 26 min, 40 s.

Pigs were dived inside a transport kennel specially modified to be gastight, other than through inlet and outlet valves. Heliox (80%/20%) was flushed into the kennel at flow rates up to 100 l/min until a total volume of 500 l was flushed through the box. In a preliminary experiment, a fixed volume of 500 l of heliox was flushed through the kennel and measured by a flowmeter, to insure the procedure produced an accurate flush. Duplicate gas samples of 1.0 l each were then checked for percentage of N_2 by gas chromatography (Shimadzu GC14A Molsieve column), demonstrating 4.6 ± 0.8%.

After the initial flush, the kennel was then placed in the chamber, and the chamber pressurized on air while maintaining a positive flow of heliox into the kennel. This was intended to insure that the pigs breathed heliox throughout the dive, while preserving the use of the heliox mix.

Gas-Switch Dives

All procedures for the gas switch dives were identical to control, except that once 170 fsw was reached during decompression, the gas mixture flowing into the kennel was switched to air. A total of 32 pigs underwent the gas-switch dive. The adequacy of the switching procedure was checked on 2 dives after the study was completed. For these 2 dives, duplicate gas samples were taken by bomb collection (v = 500 ml) on the bottom and at the first stop. In the second dive, duplicate gas samples were also taken at the second stop. Analysis by gas chromatography revealed less than 2.0% N_2 on the bottom, with a residual 40.9% of He (after the switch) after 7 min at the first stop, and 7.8% after 10 min at the second stop, respectively.

Each day, a matched litter pair of animals was randomized by coin toss to either the control or the gas switch profile. The principal investigator was blinded to the profile dived by individual pigs. Diagnosis of neurological and cutaneous DCS was therefore made without the knowledge of the preceding dive profile.

Postdive

On surfacing, all pigs were transferred from the chamber into the laboratory observation pen where they were closely observed. Behavioral features and constitutional signs such as lethargy were noted but, not considered alone to be DCS. Neurological signs such as limb weakness, paralysis, or ataxia were recorded and considered neurological DCS. Ataxia was defined as any unsteadiness, swaying, stumbling, or misplacement of limbs in the absence of evident weakness. Skin DCS was also recorded. If any signs of distress were observed, pigs were sedated by diazepam (5-10 mg IV) and observation was continued until 1 h postdive, whereupon a final assessment of skin DCS was made. At this point, affected pigs were first

anesthetized by IV injection of ketamine (400 mg in 4 ml) and xylazine (20 mg in 1 ml) via the ear vein catheter, then euthanized by bolus IV injection of 30-50 ml of 4 M potassium chloride solution.

If a pig failed to develop subjective neurological signs after 1 h of observation it ran on the treadmill, where its gait was assessed. Pigs with no discernible gait abnormality were categorized as "no neurological DCS". These pigs took no further part in the protocol.

Grading Severity of Neurological and Skin DCS

In previous studies using this model, the above procedures allowed the severity of neurological DCS to be crudely graded (7). We were unable to use the same criteria in this study because most of the DCS was ataxia and there were very few deaths. However, it was noted that some cases of ataxia were transient, while other cases lasted the entire hour of postdive observation. We therefore graded severity as "severe" for persistent ataxia, and "mild" for transient ataxia. For the skin DCS, again we were unable to use the previous grading system because no lesions were larger than 20% of skin surface area.

Statistical Methods

Statistical comparison is by Yates' corrected chi-squared analysis of discrete variables in 2 ft x 2 ft contingency tables, taking $p = 0.05$ as the threshold of significance. There is no accepted way of combining statistical analysis of both incidence and severity of DCS. Each of these results is therefore considered separately. If any expected count is less than 5, Fisher's exact test is used. The study design allows for the detection of a decrease in incidence of 0.3, with a probability of Type 1 error of 0.05, and a probability of Type 2 error of 0.20, when 30 animals are used in each group.

RESULTS

Weight of Animals

The mean weight for the control group was 19.78 kg. The mean weight for the gas switch group was 19.15 kg. Although the control group had a slightly greater variance in weight, the small difference in weight between the two groups was not deemed significant.

Neurological DCS

Of the pigs dived on the control profile, 20/32 (62%) were diagnosed as suffering from neurological DCS compared to 14/32 (44%)of the pigs decompressed on the gas-switching profile. Of the 20 affected pigs in the control group, 4 had transient ataxia, 15 had persistent ataxia, and 1 died. Of the 14 affected pigs in the gas-switch group, 2 had transient ataxia and 12 had persistent ataxia. None died in the gas-switch group. Application of chi-squared analysis to compare incidence of DCS between groups (20/32 vs. 14/32) does not yield a significant difference (Yates' corrected $\chi^2 = 1.57$; $p > 0.10$). Comparing severity (16/32 vs. 12/32), chi-square also reveals no significant difference (Yates' corrected $\chi^2 = 0.57$; $p > 0.10$).

Skin DCS

Of the 32 pigs in the control group, 12 developed skin DCS, none of them covering more than 20% of skin surface area, compared to 4/32 in the gas switch group, a statistically significant difference ($\chi^2 = 4.08$; $p < 0.05$).

DISCUSSION

Compared to pigs decompressed entirely on heliox, those switched to air during decompression were less likely to develop cutaneous DCS. There was also a trend in favor of

gas switching for neurological DCS, but the trend did not reach statistical significance with our animal numbers. Also, there was no statistically significant difference in the risk of developing severe neurological DCS between the two groups. Thus, the effect of gas switching in pigs appears to be less pronounced than that of gas switching in rats and approaches the more equivocal results of the limited human trials.

It is likely that there is a measurable decrease in the risk of neurological DCS with gas switching in larger animals, but that the magnitude of the decrease is less than that for rats. It is also likely that this decrease is below the detection limit of the present experimental design. If the trend in this study continued, 64 pairs of animals would be required to reach a statistically significant difference ($\chi^2 = 4.518$; $p < 0.05$). It may also be observed that if a Yates' correction were not used on the results for neurological DCS, the result would be statistically significant for the 32 pairs used in the present study. Some have argued that the Yates' correction is employed too liberally and leads to overly conservative conclusions. However, it should be noted that the chi-square test is an approximation. In the present case if we apply an exact test such as the Fisher's, then $p = 0.105$. The same result is obtained by calculating exact 95% confidence limits on the binomial for the control profile, 20/32 (13.98-25.25) (7). It would appear that the Yates' correction is not overly conservative in the present case.

One reason gas switching may have produced a greater change in skin DCS than in neurological DCS is that skin DCS may be a more sensitive indicator of adequate decompression. In the one previous study using this model, which quantitated both cutaneous and neurological DCS, differences for skin DCS were greater than those for neurological DCS (8). In another study of this model, histopathological evidence of neurological damage occurred in 3% of pigs

that had no clinical signs of a neurological injury (9). In the case of gas switching, there may be enough of an effect to be noticed by changes in the incidence of skin DCS, but not enough to be detected by changes in the incidence of the apparently less sensitive neurological DCS.

It is perhaps more difficult to explain the difference in the effect of gas switching among rats, pigs, and humans. In the present study, it was retrospectively discovered that the switching procedure was suboptimal, since it was only after coming to the second stop that the residual percentage of He indicated a more complete switch. It is conceivable that an apparatus that allows a quicker shift to air would have a more profound effect. In the rat experiments, the shift to air was very rapid and complete. Such a limitation in our apparatus, however, still does not completely explain species differences. In the method used by Lillo, switching to air after a heliox dive also had residual He as high as 30% 15 min postswitch (2). The human data, which shows a less profound effect, had a nearly instantaneous switch, since the divers removed their face masks and began breathing from a SCUBA regulator (3). Additionally, in the present study the staged decompression allows for a greater sensitivity to the effects of gas switching, because the longer time allows a more complete turnover of the gas mixture.

One possibility for species differences may be due to the explosive decompression profile used in the rat study. Another may be a difference in tissue gas kinetics among pigs, rats, and humans. Perhaps there is a fundamental difference in the way rat tissues respond to the gas phase. A conclusion regarding these possibilities is beyond the scope of the present study. It should be further noted that both the pig and the rat model are severe models of DCS. The results obtained may not be directly comparable to humans.

In summary, gas switching was found to have a benefit in the risk of cutaneous DCS in pigs and an insignificant trend toward benefit for neurological DCS. Skin DCS appears to be a more sensitive indication of relative risk in pigs. The benefit in pigs does not appear to be as large as that in rats. Further work in humans will be necessary to confirm its benefits in operational diving.

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